

Transmission of prion diseases by blood transfusion

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Attempts to detect infectivity in the blood of humans and animals affected with transmissible spongiform encephalopathies (TSEs or prion diseases) have often been inconclusive because of the limitations of cross-species bioassays and the small volumes of blood that can be injected by the intracerebral route. A model has been developed for the experimental study of TSE transmission by blood transfusion using sheep experimentally infected with bovine spongiform encephalopathy (BSE) or natural scrapie as donors and susceptible scrapie-free sheep as recipients. Donors and recipients of the same species greatly increase the sensitivity of the bioassay and in sheep large volumes of blood can be injected by the intravenous (i.v.) route. Transmission of BSE to a single animal using this approach was reported recently. This study confirms this result with a second transmission of BSE and four new cases of transmission of natural scrapie. Positive transmissions occurred with blood taken at pre-clinical and clinical stages of infection. Initial studies indicate that following such infection by the i.v. route, deposition of the abnormal prion protein isoform, PrP^{Sc}, in peripheral tissues may be much more limited than is seen following oral infection. These results confirm the risks of TSE infection via blood products and suggest that the measures taken to restrict the use of blood in the UK have been fully justified.

Introduction

Creutzfeldt–Jakob disease (CJD) is one of a group of related diseases known as prion diseases or transmissible spongiform encephalopathies (TSEs), a group that also includes scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle. A new variant of CJD (vCJD) in human beings in the UK (Will *et al.*, 1996) is thought to have been the result of infection with the same agent that causes BSE in cattle (Bruce *et al.*, 1997). The numbers of vCJD-infected people remain unknown, although, to date, over 100 clinical cases have been recorded in the UK. Amongst many sources of concern, one major question relates to the safety of blood transfusions and blood products – especially when inadvertently sourced from individuals during the long pre-clinical phase of vCJD, a time at which these individuals may act as asymptomatic carriers of the infectious agent. There is no epidemiological evidence to indicate that iatrogenic CJD has ever occurred via blood or blood products but vCJD is a new disease with a different pathogenesis and may present different risks.

The TSE disease-associated form of the prion protein

(PrP^{Sc}) of the neuronal glycoprotein PrP^C is often used as a marker for infectivity. Using a sensitive Western blotting technique, no PrP^{Sc} was detected in the buffy coat from one vCJD patient (Wadsworth *et al.*, 2001). Although a novel method for detection of PrP^{Sc} in scrapie sheep blood has been described (Schmerr *et al.*, 1997), the study was limited by a low number of samples and the technique requires further validation. Other investigators using the more conventional method of immunocytochemistry failed to demonstrate PrP^{Sc} in peripheral blood leucocytes of scrapie-infected sheep (Herrmann *et al.*, 2002).

An alternative to PrP^{Sc} detection is direct bioassay of infectivity by inoculation of material into hosts of the same or different species. In laboratory rodents experimentally infected with TSE, a number of investigators have demonstrated infectivity in blood and blood components during the pre-clinical and clinical phases of infection (Brown *et al.*, 1998; Diringier, 1984; Manuelidis *et al.*, 1978). However, infectivity has not been isolated, so far, from blood components of natural animal hosts of TSEs (Hadlow *et al.*, 1982; Marsh *et al.*, 1973). Isolated reports of transmission of CJD to laboratory rodents by whole blood or buffy coat from human patients have been questioned for a variety of reasons (Brown, 1995). A large-scale study conducted by the National Institutes of Health

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failed to demonstrate infectivity in blood from 13 patients with CJD, using either highly susceptible primates or rodents as bioassay hosts (Brown *et al.*, 1994). With vCJD, no infectivity was detected in blood from two patients using mouse bioassays (Bruce *et al.*, 2001). Many of these studies could have failed to reveal low levels of infectivity in blood because of the use of rodents as bioassay hosts, thus limiting the sensitivity by crossing a species barrier. Also, in most cases, the intracerebral (i.c.) route of inoculation was used, because it is the most efficient, but this severely limits the volume of blood that can be assayed. Thus, where transmission from blood has been successful, infectivity was usually concentrated in some way, for example, by the use of buffy coat fractions. Transmission by the intravenous (i.v.) route has been shown to be up to seven times less efficient than following i.c. infection (Brown *et al.*, 1999), but there have been very few attempts to transmit TSEs by whole blood transfusion. Units of whole blood from three CJD cases were transfused into chimpanzees with negative results (Brown *et al.*, 1994) and pooled blood from three terminally ill TSE-infected mice produced disease in 1 of 20 transfusion recipients (Brown *et al.*, 1999).

Sheep infected orally with BSE show widespread deposition of PrP^{Sc} in the lymphoreticular system (LRS) (Foster *et al.*, 1996a, 2001b), similar to that seen in human vCJD patients. In contrast, in cases of sporadic human CJD and cattle BSE, peripheral pathogenesis does not appear to involve the LRS (Hill *et al.*, 1999; Wells *et al.*, 1998). Sheep were chosen as a model in which to study transmission of TSEs by blood transfusion because of the similarity of the pathogenesis with vCJD and because large volumes of blood can be transferred in the absence of a species barrier.

We have transfused whole blood and buffy coat from BSE-infected sheep and natural scrapie-infected sheep into susceptible but scrapie-free recipient animals. In the first report on these experiments (Houston *et al.*, 2000), we described a single case of BSE infection via blood transfusion. The significance of this finding in a single animal has been questioned. However, the present report gives details of further successful transmissions from BSE and natural scrapie cases, the latter being the first conclusive demonstration of infectivity in blood of naturally infected individuals. Although still incomplete, our study indicates a frequency of transmission of TSEs in at least 10% of the transfusion recipients. We have decided to provide an update of our results because of the potential importance of the study for human health. In addition, in the two BSE transfusion cases examined so far, deposition of PrP^{Sc} in peripheral tissues appears rather limited when compared with sheep infected by the oral route. The potential implications of this observation for pre-clinical diagnosis and screening are discussed.

Methods

■ **Sheep.** The sheep were all Cheviots and were (as indicated) either from the Edinburgh NPU Cheviot flock (blood donor animals) or from the

DEFRA scrapie-free (DEFRA/SF) flock of New Zealand (all blood recipients and scrapie-negative control donors). DEFRA/SF animals are exactly the same as those known previously as MAFF/SF and simply reflect the change of name of the Ministry of Agriculture, Fisheries and Food (MAFF) to Department of Environment, Food and Rural Affairs (DEFRA) in 2001. This flock was established from sheep imported from New Zealand in 1998 and is maintained in strict isolation from UK livestock and any other potential source of infection on land formerly used exclusively for growing crops. A large spectrum of PrP genotypes are produced and maintained in the flock, with some sheep of the sensitive genotypes being kept to older age as biological sentinels. Cull animals are routinely screened for evidence of TSE infection and there have been no recorded cases of scrapie since the flock was established. Recipient sheep were housed in a purpose-built isolation unit on a separate site (Compton, UK) around 400 miles away from the infected blood donors and completely isolated from other UK sheep. BSE and scrapie transfusion recipients, and positive and negative control animals, were housed continuously in separate pens so that they never came into direct contact with one another. Precautions were taken to minimize the risks of cross-contamination between the groups: for example, use of dedicated equipment for each group. The animals were fed on a diet of dried lucerne pellets and hay, with free access to a mineral supplement. Care was taken to ensure that the diet did not contain any animal protein.

Sheep have one of the most complex blood grouping systems known (Tizard, 1992) and therefore exact cross-matching of donors and recipients was not possible. Single transfusions only were made between animals that were checked for compatibility using a simple agglutination test (mixing recipient serum and donor erythrocytes and vice versa).

Genotypes of all sheep were confirmed by sequencing the region coding of the PrP gene (Foster *et al.*, 2001a) and are presented in three-codon format with each codon (136, 154 and 171) in turn for each allele in turn. The single letter amino acid code is used as follows: A, alanine; H, histidine; R, arginine; Q, glutamine; V, valine; X, any of the known polymorphisms at that position.

■ **BSE blood transfusions.** The experimental BSE incubation period is controlled in sheep by the PrP gene codons 136 and 171, with no major effect of codon 154. BSE has the shortest incubation period in AXQ/AXQ sheep and extremely long incubation periods in heterozygote animals expressing AXQ with the ARR or VRQ alleles (Foster *et al.*, 2001a and unpublished observations). Of the 18 donor sheep (all genotype AXQ/AXQ), 17 were taken from the NPU Cheviot flock 'negative' line (not affected by natural scrapie) and were infected with 5 g cattle BSE brain homogenate by the oral route as part of a separate series of experiments. Five of the donor animals developed BSE at 559–761 days (mean \pm SD = 640 \pm 78 days) post-challenge, three are still alive at more than 1500 days post-challenge; however, the other donors ($n = 9$) were culled during the pre-clinical phase as part of a serial cull pathogenesis experiment.

Due to a shortage of clinical BSE cases in the orally infected group, an ARQ/ARQ Cheviot sheep (DEFRA/SF) that was showing clinical signs following intracerebral inoculation of 0.05 g cattle BSE brain homogenate (incubation period 671 days) was used to provide blood for the final transfusion in the group.

The 24 recipients for the BSE blood transfusions were sheep of ARQ/ARQ genotype from the DEFRA scrapie-free flock of New Zealand origin (DEFRA/SF sheep). Six donors were used to provide both whole blood and buffy coat transfusions but these were transfused into separate recipient sheep.

■ **Natural scrapie transfusions.** Natural scrapie occurs in the NPU Cheviot flock positive line in carriers of the VRQ allele (Hunter *et al.*, 1996) and the 11 donors from this group were of VRQ encoding

genotypes: ten VRQ/VRQ and 1 VRQ/ARQ. Transmission is believed to occur perinatally and clinical cases develop at 2–3 years of age. Four of the donors developed natural scrapie at 1080–1484 days of age, five are still alive at 700–1081 days of age and two were culled for welfare reasons before signs of scrapie were expected (349 and 975 days of age). The 21 recipients for the natural scrapie blood transfusions were sheep of VRQ/VRQ genotype from the DEFRA/SF flock. Ten donors donated blood for both whole blood and buffy coat transfusions but single transfusions per recipient were carried out.

■ Negative control transfusions. Negative controls for the BSE blood transfusions consisted of seven healthy donors of AXQ/AXQ genotype of ages ranging from 497 to 1676 days from the NPU Cheviot flock negative line. They provided blood and/or buffy coat for transfusion into 12 ARQ/ARQ genotype sheep from the DEFRA/SF flock. None of the donors has developed TSE to date, although two were culled at 580 days of age as part of a serial cull pathogenesis study. One sheep died of intercurrent disease (not scrapie) at 865 days of age and the other four donors are alive at the time of writing, at 1096–2170 days of age.

To act as negative controls for the natural scrapie blood transfusions, eight VRQ/VRQ genotype sheep of ages ranging from 211 to 629 days from the scrapie-free DEFRA/SF flock provided blood and/or buffy coat for transfusion into 16 VRQ/VRQ genotype animals also from the DEFRA/SF flock. None of the donor sheep has developed TSE to date and are alive and healthy at 699–821 days of age.

■ Intravenous route/positive controls. When this study was set up, it was unknown whether BSE or scrapie could be transmitted by the i.v. route. A negative result from the transfusions could have meant that the i.v. route was particularly ineffective. Positive control sheep were therefore infected by the i.v. route with BSE cattle brain homogenate, which is known to infect sheep successfully by both oral and i.c. routes. The ten challenged animals were of ARQ/AXQ genotypes and were infected by the i.v. route with the equivalent of 0.2 g BSE-affected cattle brain homogenate, obtained from DEFRA. Initially, four sheep were infected by i.v. inoculation of 2 ml 10% brain homogenate. However, following a severe adverse reaction in one animal, which was removed from the study, the remaining seven animals were inoculated by the i.v. route with 20 ml 1% brain homogenate and treated with a non-steroidal anti-inflammatory drug, ketoprofen (Ketofen, 10%), before inoculation to try to prevent shock.

■ Blood transfusion method. Blood was collected from the jugular vein of sedated sheep into 500 ml sterile blood transfusion bags containing sodium citrate. From donors that were about to be necropsied, two units of whole blood (900 ml total) were collected. From donors that were to be left alive until clinical signs developed, separate collections of a single unit of blood were made at least 28 days apart. In either case, one unit was transfused as whole blood and the other was used to prepare the buffy coat. Buffy coat fractions were produced by spinning down whole blood and withdrawing the white cell component under aseptic conditions, according to standard protocols employed by the Scottish National Blood Transfusion Service. Whole blood packs were stored at 4 °C (buffy coats were stored at 20 °C) prior to use, usually on the following day (always within 3 days of collection). Blood was given to transfusion recipients via a single-use disposable blood administration set and an i.v. catheter placed in the jugular vein, at a rate of approximately 200–300 ml/h.

■ PrP^{Sc} detection by immunocytochemistry and Western blot. PrP^{Sc} immunocytochemistry was carried out as described pre-

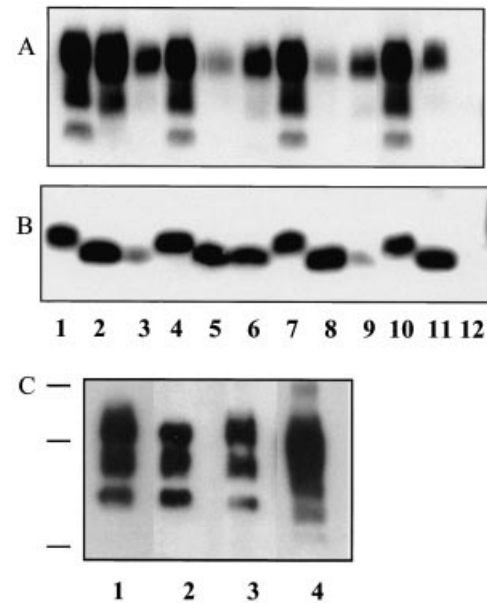


Fig. 1. Western blot detection of PrP^{Sc} in sheep brain. (A) Proteinase K-treated and (B) PNGase-treated samples from individual sheep. (A, B) The same sheep in each lane are indicated. Lanes: 1, 4, 7 and 10, natural scrapie; 2, 3, 5, 6, 8, 9, 11 and 12, experimental BSE; 2 and 9, i.c. challenge; 3 and 11, oral challenge; 5 and 6, transfusion cases D505 and F19, respectively; 8, D505's blood donor J2599 at terminal stage; 12, F19's blood donor J2746 at pre-clinical stage. (C) Proteinase K-treated individual scrapie sheep brain samples showing a natural scrapie transfusion case. Lanes: 1, natural scrapie; 2, F141's blood donor 59x27 at clinical phase; 3, transfusion case F141; 4, experimental BSE case challenged by the i.c. route.

viously (Foster *et al.*, 2001b). The primary antibody, BG4, was a monoclonal antibody (mAb) raised in mice against recombinant bovine PrP, binding to epitopes in the regions of aa 47–57 and 89–99 (courtesy of TSE Resource Centre, IAH, Compton, UK). The secondary antibody was biotinylated rabbit anti-mouse IgG, which was linked to streptavidin peroxidase with 3-amino-9-ethylcarbazole as the chromogen. Comparisons of PrP^{Sc} distribution were made with sheep infected with BSE (by oral or i.c. routes) and with animals naturally infected with scrapie in the NPU Cheviot flock, using methods published previously (Foster *et al.*, 2001a, b).

For Western blotting to detect PrP^{Sc}, extraction procedures were used as described previously (Hope *et al.*, 1986; Towbin *et al.*, 1979). PNGase-treated PrP^{Sc} was prepared using the method described by Collinge *et al.* (1996). For Fig. 1(A, B), mAb 6H4 (Prionics) was used to detect of PrP^{Sc} and was visualized with a chemiluminescence substrate (Roche). For Fig. 1(C), mAb 8H4 was used (Zanusso *et al.*, 1998).

Results

Blood transfusions

To date, 24 transfusions from BSE-challenged sheep have been carried out, including seven with buffy coat preparations and 17 with whole blood. Two of the 24 recipients have so far been culled after showing clinical signs typical of TSE in sheep. The first of these (D505) was reported previously (Houston *et*

al., 2000) and occurred 610 days following transfusion with whole blood taken from a donor animal (J2559) during the pre-clinical stage of infection (50% of incubation period). The second case (F19) is new and also received whole blood that was taken from the donor (J2746) during the pre-clinical stage of disease, at 282 days following oral BSE challenge. The donor was culled at this time as part of another experiment; however, 282 days represents 45% of the mean BSE incubation period in this study. The recipient (F19) developed BSE with an incubation period of 538 days. Two additional animals are currently showing early signs of BSE, having been transfused with whole blood taken when the donors were themselves at the clinical phase of BSE. Of the remaining transfused sheep, there has been one intercurrent death (not TSE) at 1108 days post-transfusion and the other 19 animals are between 68 and 1243 days post-transfusion and remain healthy at the time of writing. If the BSE suspect cases are confirmed, this brings the minimum rate of infection by transfusion in this part of the study to 4 of 24, roughly 17%. Four sheep that received blood or buffy coat from confirmed BSE cases are still alive and free from clinical signs at the time of writing (at 68, 499, 562 and 1216 days post-transfusion).

Of the 21 animals transfused with blood taken from natural scrapie-infected animals, four animals have been culled with clinical signs of scrapie to date. Blood was collected from the donors (61x75, 61x68, 59x27 and 59x28) at time-points of 761, 764, 1138 and 1080 days of age. The donors developed clinical signs of scrapie at 1324, 1112, 1484 and 1080 days of age, respectively, making the blood donations at 57, 69, 77 and 100% (clinical phase) of donor survival time, respectively. Scrapie developed in recipients (F144, F153, F141 and F143) at 614, 624, 571 and 737 days post-transfusion, respectively. Of the 17 remaining recipients, one died of unrelated causes (not TSE) at 364 days post-transfusion and the others remain healthy, although 13 animals are at < 600 days post-transfusion and could still potentially develop scrapie. Three of the scrapie-positive transfusions made with whole blood came from donors that also donated buffy coat samples for transfusion into different recipients, which all remain healthy at 701 days post-transfusion. However, one positive transmission occurred in a sheep (F143) transfused with buffy coat taken at the clinical end-point from the donor, showing that this blood fraction can transmit infection, at least at the clinical phase of disease. Unfortunately, insufficient blood was collected from this donor to perform a whole blood transfusion in another recipient for comparison of incubation periods. This part of the study gives a minimum transmission rate by blood transfusion of 4 of 21 or 19% for natural scrapie.

The negative controls for these experiments are currently at slightly earlier stages post-transfusion, the result of limited availability of DEFRA/SF sheep. The 12 negative controls for the BSE study are alive and healthy at 482–903 days post-transfusion. There has been one intercurrent death (not TSE) among the negative controls for the scrapie study at 464 days

post-transfusion. The other 15 recipients are alive and healthy in two groups at 38–103 ($n = 6$) and 516–537 ($n = 9$) days post-transfusion. It should be noted that both the BSE- and scrapie-negative controls are at a relatively early stage of the possible incubation time and we must wait until the study is complete before it can be confirmed that the controls are free of TSEs.

Positive controls were given a dose of 0.2 g BSE-affected cattle brain homogenate by the i.v. route to provide evidence that the i.v. route allows infection by TSE challenge in sheep, not established previously. Of ten animals (AXQ/AXQ genotype) challenged by the i.v. route, two animals were culled with clinical signs of BSE at 605 and 655 days post-challenge and three additional animals are showing early clinical signs at the time of writing, at 626–702 days post-challenge.

PrP^{Sc} detection by Western blot

Glycoform analysis of PrP^{Sc} by Western blot is one means by which strains of TSEs may be identified (Collinge *et al.*, 1996). PrP^{Sc} samples were prepared from the brains of affected sheep for analysis and comparison with animals known to be infected with BSE or scrapie. Fig. 1(A, lanes 5 and 6) shows PrP^{Sc} extracted from the BSE transfusion cases D505 and F19, respectively. The glycoform pattern is dominated by the high molecular mass isoform of PrP^{Sc}, as expected for BSE. For comparison, proven transmissions of BSE to sheep in Fig. 1(A, lanes 2 and 9) (i.c. challenge) and Fig. 1(A, lanes 3 and 11) (oral challenge) show characteristic predominance of high molecular mass glycoforms. The donor for D505 survived until clinical signs of BSE developed. The Western blot also shows the BSE glycoform pattern in the donor (J2599) at clinical phase (Fig. 1A, lane 8). The donor for F19 (J2746) was culled at the time of blood donation, 282 days post-challenge, in the absence of clinical signs of BSE. A brain sample taken from J2746 at the same time as the blood donation showed no sign of PrP^{Sc} (Fig. 1A, lane 12). In contrast, natural scrapie PrP^{Sc} (Fig. 1A, lanes 1, 4, 7 and 10) shows a clearly different glycoform pattern typical of UK natural scrapie (Hope *et al.*, 1999).

An additional characteristic of BSE is the 'band shift' seen in the lowest, unglycosylated isoform of PrP^{Sc}, which is of lower apparent molecular mass than that seen in natural scrapie (Hope *et al.*, 1999; A. Chong, unpublished observations). As this feature was not clear from the blots in Fig. 1(A), PNGase treatment was used to remove the carbohydrates from the PrP^{Sc} protein. As shown in Fig. 1(B), in all BSE cases, the resulting band sizes have a slightly lower apparent molecular mass than is seen in natural scrapie.

Fig. 1(C) shows the glycoform analysis of one of the scrapie blood transfusion cases (F141) (Fig. 1C, lane 3) and the corresponding donor sheep (59x27) at clinical phase (Fig. 1C, lane 2), compared with another natural scrapie case (Fig. 1C, lane 1) and an experimental sheep BSE case (Fig. 1C, lane 4).

Table 1. Comparative immunostaining of PrP^{Sc} in the brain and some lymphoid tissues of sheep experimentally infected with BSE (transfusion: i.v., i.c. and oral) and cases of natural scrapie

Incubation period is shown in days. Age at death (days), where necessary, is indicated in bold. Immunocytochemistry staining results are indicated as negative (–), marginal (+), definite (++) or strong (+++).

Route of challenge	Sheep no.	Incubation period	PrP genotype	PrP ^{Sc} immunocytochemistry					
				Dorsal motor vagus nucleus	Thalamus	Tonsil	Mesenteric lymph node	Peyer's patches	Spleen
BSE Blood transfusion	1 (D505)	610	ARQ/ARQ	++	++	+	–	+	+
	2 (F19)	536	AHQ/AHQ	+++	+	–	+	+	+
Intracerebral	3	504	ARQ/ARQ	+++	+++	–	–	–	–
	4	495	ARQ/ARQ	+++	+++	–	–	–	–
Oral	5	596	ARQ/ARQ	+++	+++	+++	+++	+++	+++
	6	935	AHQ/AHQ	+++	+++	+++	+++	+++	+++
Scrapie Natural	7	1080	VRQ/VRQ	+++	+++	+++	+++	+++	+++
	8	1112	VRQ/VRQ	+++	+++	+++	+++	+++	–

The transfusion case clearly shows a PrP^{Sc} pattern typical of scrapie rather than that of BSE. Murine transmissions are being set up to confirm the identity of the TSE agent in the transfusion cases but the genotypes of affected animals (ARQ/ARQ for BSE and VRQ/VRQ for scrapie) and the PrP^{Sc} Western blot patterns strongly support the conclusion of successful transmission of both BSE and scrapie by blood transfusion.

Immunocytochemistry

The distribution and intensity of PrP^{Sc} immunostaining in two of the BSE transfusion cases has been compared with our extensive data on sheep infected with BSE by other routes (oral and i.c.) and with natural scrapie. For simplicity, only two sheep from each group are described (Table 1). Selected areas of the brain were scrutinized for PrP^{Sc} immunostaining, including the dorsal motor nucleus of the vagus (DMNV) in the medulla oblongata and thalamic nuclei of the diencephalon, which we have shown previously to stain positively in natural scrapie (Foster *et al.*, 1996b) and in sheep orally dosed with BSE (Foster *et al.*, 2001b). Peripheral lymphoid tissues were also collected, although reporting here has been restricted to the tonsil, spleen, mesenteric lymph node and Peyer's patches of the gut.

The DMNV showed strong PrP^{Sc} immunostaining in six sheep, two infected with BSE by blood transfusion, two infected with BSE by the oral route and two infected with natural scrapie (Table 1), but staining was reduced slightly in both of the BSE cases inoculated by the i.c. route. It was especially prominent as perineuronal and extracellular punctate staining, with the cell body clearly outlined (Fig. 2). In contrast, the thalamus demonstrated fairly weak immunostaining in both transfusion cases but more pronounced staining was seen in the natural scrapie cases and also in one each (Table 1, sheep 4 and 6) of the oral and i.c. infected BSE cases.

PrP^{Sc} immunostaining was variable in peripheral lymphoid tissues. For example, both BSE blood transfusion cases showed nil or marginal staining of the tonsil (Fig. 3) and mesenteric lymph node (Fig. 4 and Table 1), as well as retropharyngeal, submandibular and ileo-caecal lymph nodes (data not shown), in contrast to the BSE oral and natural scrapie cases. However, Peyer's patches in the proximal colon stained strongly in one transfusion case (Table 1, sheep 1) and less so in the other. Jejunal Peyer's patches only stained lightly (data not shown) in both sheep.

Both orally dosed BSE cases (Table 1, sheep 5 and 6) demonstrated clearly observable immunostaining in all peripheral lymphoid areas tested, with sheep 6 having strong tonsillar staining (Fig. 5). Both natural scrapie cases (Table 1, sheep 7 and 8) had intense immunostaining in the mesenteric lymph node (Fig. 6) and tonsil, as well as retropharyngeal, ileo-caecal, prescapular and submandibular lymph nodes (data not shown).

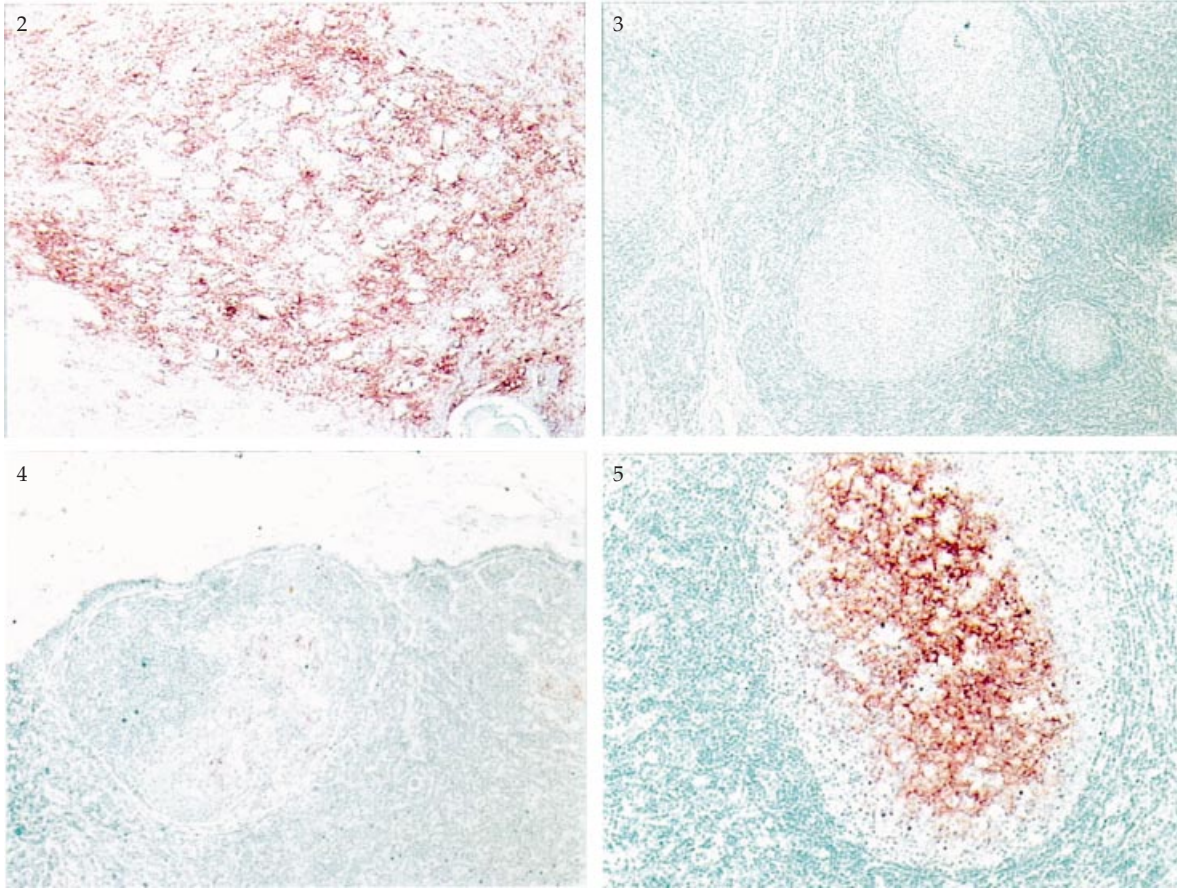


Fig. 2. Transfusion BSE case. PrP^{Sc} immunostaining in the DMNV shown in sheep 2 at an incubation period of 536 days post-transfusion. Magnification, $\times 10$.

Fig. 3. Transfusion BSE case. No PrP^{Sc} immunostaining in lymphoid tissue from the palatine tonsil of sheep 2 at an incubation period of 536 days post-transfusion. Magnification, $\times 10$.

Fig. 4. Transfusion BSE case. Marginal PrP^{Sc} immunostaining in lymphoid tissue from the mesenteric lymph node of sheep 2 at an incubation period of 536 days post-transfusion. Magnification, $\times 20$.

Fig. 5. Oral BSE case. PrP^{Sc} immunostaining in lymphoid tissue from the palatine tonsil of sheep 6 at an incubation period of 935 days post-infection. Magnification, $\times 20$.

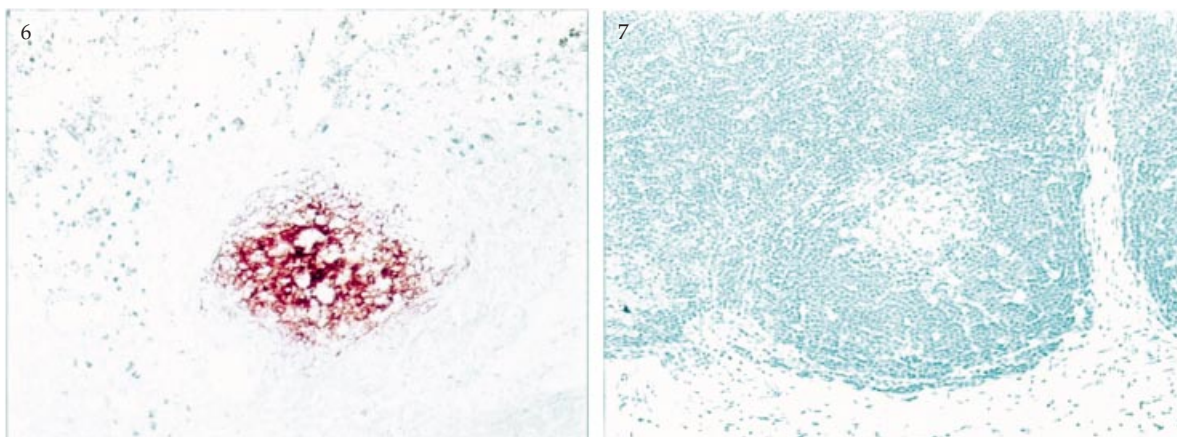


Fig. 6. Natural scrapie case. PrP^{Sc} immunostaining in the mesenteric lymph node of sheep 7, which developed natural scrapie at 1080 days of age. Magnification, $\times 20$.

Fig. 7. Intracerebral BSE case. No PrP^{Sc} immunostaining in the mesenteric lymph node of sheep 3 at an incubation period of 504 days post-infection. Magnification, $\times 20$.

Sheep challenged by the i.c. route showed no PrP^{Sc} immunostaining in any of the lymphoid tissues examined (Fig. 7).

Discussion

With this report we have confirmed and extended our initial observation of a single case of BSE following transfusion of blood from a BSE-infected sheep and have provided the first conclusive evidence of significant levels of infectivity in blood in a naturally occurring TSE (scrapie). The experiment may take up to 5 years to complete; however, so far we have clear evidence of disease transmission by the blood transfusion route in 2 of 24 sheep (8%) with BSE and 4 of 21 sheep (19%) with scrapie, with two additional animals showing clinical signs in the BSE group. If the clinically suspect BSE-transfused sheep progress as expected, this would bring the transmission rate for BSE up to 17%, comparable with the scrapie rate. Positive transmissions have occurred not only with samples taken from sheep at the clinical phase of disease but also with those from apparently healthy donors as early as halfway through the incubation period (Fig. 1, lane 9; no PrP^{Sc} detection in the brain of donor J2746). Each TSE is transmitting to its appropriate susceptible genotype (AXQ/AXQ for BSE and VRQ/VRQ for scrapie) and Western blot/glycoform analyses support the conclusion that donors and recipients are infected with the same strains of BSE and scrapie. Our negative controls remain healthy, although still at relatively early stages post-transfusion and our positive controls are developing clinical signs at around, or greater than, 600 days post-challenge, showing incubation periods very similar to the transfusion cases.

Whole blood transfusion (400–450 ml) cases are presenting incubation periods of around 600 days, which is very similar to those resulting from i.v. injection of 0.2 g BSE cattle brain homogenate. The transfusions might be expected to be more efficient because they are a sheep-to-sheep transmission with no species barrier, which contrasts with the i.v. brain infections, which is a cattle-to-sheep transmission. A full titration of the inoculum used in the cattle BSE brain i.v. controls is under way in mice but is incomplete at the time of writing. Accurate estimation of the levels of infectivity in blood will require i.v. titration in sheep; however, the results presented here suggest that they are significantly higher than suspected previously. Another important consideration is the distribution of infectivity among different blood components. Perhaps surprisingly, most positive transmissions so far have followed transfusion of whole blood rather than buffy coat, whereas previous studies have tended to find infectivity concentrated in the buffy coat fraction. As we now have a clinical case of scrapie resulting from transfusion of buffy coat, it is clear that, in our model, infectivity is also carried by the cells in this fraction. However, these preliminary results suggest that infectivity is not confined to the buffy coat fraction and that

there may also be significant levels of infectivity in the plasma and/or red cell fractions.

The presence of infectivity in blood suggests that it should be possible to detect PrP^{Sc} or other surrogates of infectivity by alternative methods, with obvious benefits for development of ante-mortem diagnostic tests. Early reports of the use of capillary electrophoresis to detect PrP^{Sc} in the blood of scrapie-infected sheep showed some promise (Schmerr *et al.*, 1997); however, a recent study could not detect PrP^{Sc} in peripheral blood leucocytes of scrapie-infected sheep using immunocytochemistry (Herrmann *et al.*, 2002). PrP^C is known to be expressed only on peripheral blood mononuclear cells in sheep, in contrast to humans where it is also found on platelets and, at low levels, on erythrocytes (Barclay *et al.*, 2002; Herrmann *et al.*, 2001; Holada *et al.*, 1998). Since tissues that express PrP^C do not always equate with areas that accumulate PrP^{Sc} and infectivity during disease, the distribution of infectivity in blood fractions of different species clearly merits more detailed analysis.

Immunocytochemical detection of PrP^{Sc} in peripheral tissues of two of the BSE transfusion cases has shown a greatly reduced involvement of lymphoid tissues, including tonsil, in the peripheral pathogenesis compared with NPU Cheviot sheep orally infected with BSE or natural scrapie (Foster *et al.*, 2001a). A recent report has shown that a proportion of Romney sheep in the late pre-clinical stages of infection with BSE following oral dosing (22 months post-infection) have PrP^{Sc} deposits in the CNS in the absence of any detectable involvement of peripheral lymphoid tissues (Jeffrey *et al.*, 2001). This study also noted the relatively late and variable onset of PrP^{Sc} accumulation in the lymphoid tissues of BSE-infected sheep. A more detailed study of BSE and scrapie transfusion cases, and positive controls, will be undertaken to determine whether lack of involvement of the LRS is a consistent feature in animals infected by the i.v. route; the results will be published at a later date. If our preliminary observations are confirmed, there may be implications for human patients with the misfortune to have received blood products from vCJD cases, because a negative tonsil biopsy as a means of reassurance might very well be unreliable. On the other hand, it also may mean that if a human patient became infected with vCJD by the i.v. route, then the peripheral tissues and blood of this secondary case may not themselves be highly infectious.

In conclusion, our results so far indicate that, with more than 10% of transfusions resulting in disease in the recipients, blood transfusion represents an appreciable risk for transmission of TSEs in sheep and, by extension, of vCJD in human beings. The relatively short and consistent incubation periods seen in positive cases suggests that levels of infectivity in the blood may be higher than suspected previously, even in the pre-clinical stages of infection, and/or that transmission by the i.v. route is highly efficient. From these preliminary results, it would appear that measures taken to safeguard the blood

supply in the UK are fully justified. However, further work, in particular a thorough investigation of the distribution of infectivity in different blood fractions, is required before a reliable estimate of the risks associated with contaminated blood products can be made.

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